Gene banking of Indian major carps and breeding technique of threatened fishes

Introduction

Indian major carps (catla, rohu and mrigal) (Fig. 1 a-c) contributes 23% of total fish production (DoF, 2012). About 99% of total seeds are produced in hatcheries to meet the demand of farmers. But the quality of seed is deteriorating day by day due to inbreeding, genetic drift, negative selection, indiscriminate inter-specific hybridization, improper broodstock development etc. As a result hatchery produced seeds show low growth, high mortality, more deformities and disease susceptibility, and less fecundity. The nurserers and fish culturists are not getting expected production and profit out of their business which is hampering the total fish production. For sustainable aquaculture of these species sufficient number of genetically potential brood needs to be ensured for producing quality seeds through best hatchery management practices. To produce quality broods, fish samples from natural sources were collected and genetically characterized through allozyme electrophoresis and microsatellite DNA markers followed by selective breeding of superior stocks.

Bangladesh has 260 freshwater fish species of which 12 have been categorized as critically endangered, 28 as endangered and 14 as vulnerable (IUCN Bangladesh, 2000). These fish species became endangered due to environmental degradation and human interferences. The situation was felt critical demanding immediate address to overcome its serious consequences in fisheries sector. In these circumstances two critically endangered (mohashol, bagair) and one endangered (baim) fishes were brought under the research purview of the project with an aim of saving them from the present state of endangered situation through establishment of dependable breeding techniques (Fig. 1 d-f)

Objectives

Indian major carps (IMCs)
Improved broodstock development through selective breeding and live and cryogenic gene banking

Threatened species
Domestication and breeding technique development and conservation


Fig. 1 a *Catla catla* (catla)


Fig. 1 b *Labeo rohita* (rohu)


Fig. 1 c *Cirrhinus cirrhosus* (mrigal)


Fig. 1 d *Tor tor* (mohashol)


Fig. 1 e *Bagarius bagarius* (bagair)


Fig. 1 f *Mastacembelus armatus* (baim)

Fig. 1 Indian major carps (a-c) and threatened fishes (d-f)

Achievements of Indian major carps

Growth performance

IMCs seeds from three different rivers viz. the Halda, the Jamuna and the Padma as well as from six hatcheries of three regions namely Mymensingh (Brahmaputra and Rahim hatchery), Comilla (Rupali-C and Bismillah hatchery) and Jessore (Ma Fatema and Rupali-J hatchery) were collected and their growth performances were monitored for a period of six months.

The growth performance of the Halda rohu and mrigal were significantly ($P<0.05$) higher (102.85 ± 18.57g and 105.07 ± 15.29g respectively) than those of hatchery and other natural sources. Whereas catla of the Jamuna (191.79 ± 15.22g) showed similar growth performance when compared with the Halda (190.55 ± 15.64g) but significantly ($P<0.05$) higher than the Padma and other hatchery sources (Fig. 2).

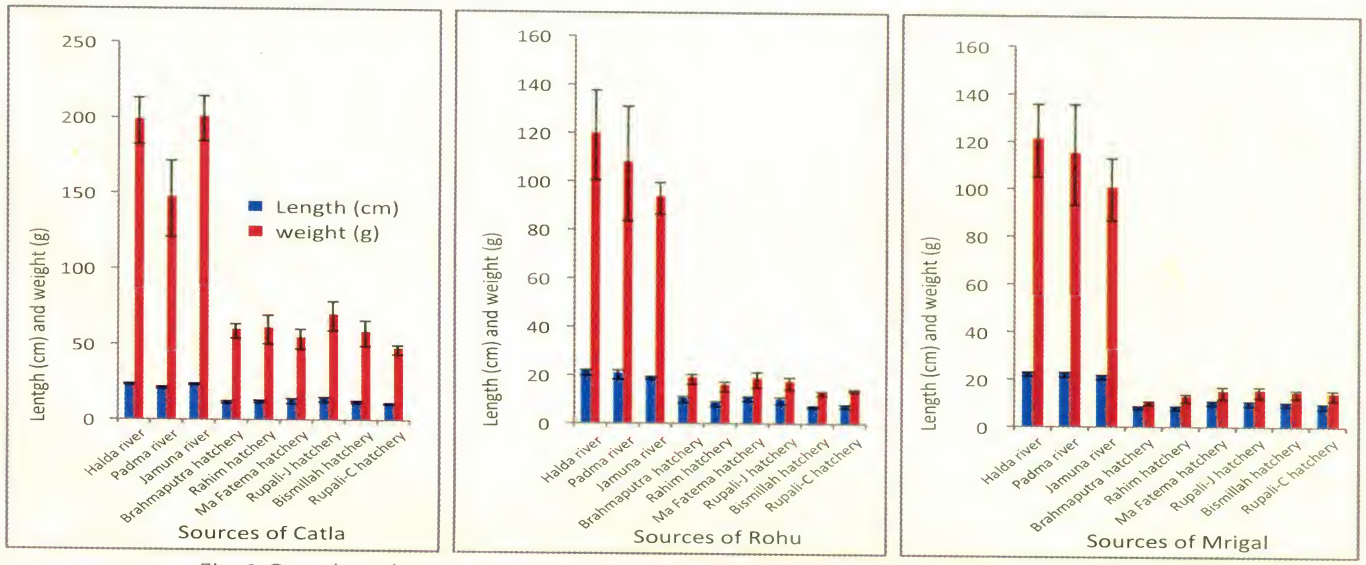

Fig. 2 Growth performance of catla, rohu and mrigal of different river and hatchery sources

Genetic analysis through allozyme electrophoresis technique

Allozyme electrophoresis was done with muscle samples from all nine sources using four enzymes (LDH, MDH, PGM and GPI) and seven presumptive loci were screened. Four loci (*Mdh-1**, *Pgm**, *Gpi-1** and *Gpi-2**) were found to be polymorphic in rohu populations and three (*Mdh-1**, *Mdh-2** and *Gpi-1**) were found to be polymorphic in case of mrigal populations. From the study it was found that the riverine and hatchery stocks formed 2 different clusters constituted by different genetic distances.

Genetic analysis through microsatellite DNA marker

Microsatellite study was conducted only with rohu samples of 3 riverine populations using 6 (*Lr3, Lr21, Lr12, Lr14b, Lr24* and *Lr26*) microsatellite DNA marker. The study revealed a low level of genetic variation at microsatellite loci within and between rohu populations (Fig. 3).

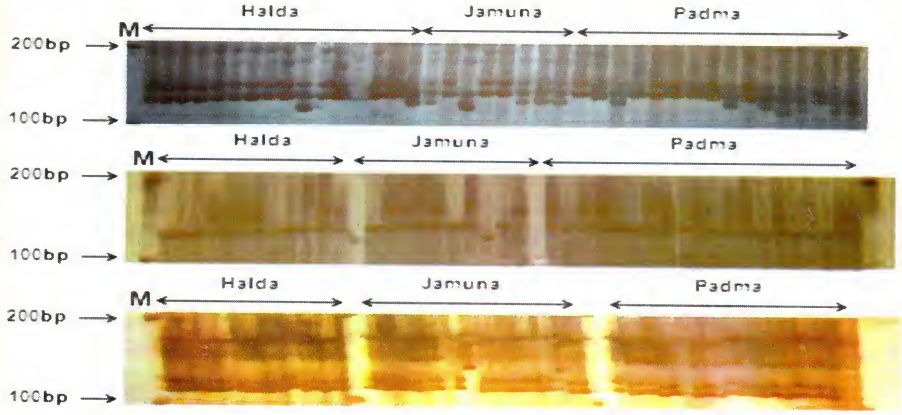

Fig. 3 Microsatellite profiles of the Halda, the Jamuna and the Padma stocks of *L. rohita* at locus *Lr3*, *Lr21* and *Lr24* respectively; M: molecular weight marker (100bp DNA ladder)

Cryopreservation of spermatozoa

A series of experiments were conducted to develop and standardize the protocols for cryopreservation of sperm of rohu and mrigal with a view to conserve gene pool. Alsever's solution with DMSO and egg-yolk citrate with methanol were found to be suitable for cryopreservation of sperm of rohu. On the other hand, Alsever's solution with DMSO and methanol were found to be suitable for mrigal spermatozoa (Fig. 4). Catla are not yet mature enough to start the cryopreservation work. We suppose to do the work in the next breeding season.

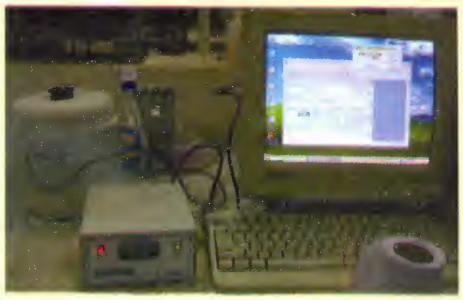

Fig. 4 Sperm cryopreservation with computer assisted controlled-rate freezer

Selective breeding

On the basis of growth performances and genetic study the Halda population was identified as better stock. For induced breeding of IMCs ready to breed 5 pairs of rohu and mrigal of the Halda stock were induced to breed using carp pituitary gland (PG) extract (Fig. 5) The fish were fully mature and 100% ovulation was occurred. Fertilization and hatching rates were found 86.46±6.7%, 77.24±4.3% respectively. Catla were not mature enough to breed this year.

Fig. 5 Collection of ovulated eggs through stripping of the Halda mrigal

Achievements of threatened species

Fish collection

One hundred and fifty mohashol were collected from the Shomeshshori river at Shushang Durgapur in Netrokona district. One hundred and twenty juveniles of bagair and 120 baim were collected from the old Brahmaputra river in Mymensingh district and haor region in Kishoreganj district respectively.

Domestication and growth study

One hundred and twenty mohashol (average weight: 1.216±0.13kg) were reared in the earthen pond of 0.12 ha area, having inlet and outlet facilities. The fish were fed with the commercial supplementary feed (Mega feed) having 30% protein at the rate of 2 to 6% of body weight. The growth (0.207±0.13kg) of mohashol was observed for a period of 6 months (August 2011- January 2012).

Thirty five bagair were stocked in each of three chambers (9.50m×1.00m area each) of the raceway having all facilities like continuous water supply and shelter. The fish were fed with 3 different supplementary feeds, i. e. Mega feed, trash fish and chicken viscera. The highest (319.30±18.17g) and the lowest growth (134.70±5.12g) were observed where trash fish and Mega feed were applied respectively for a period of 6 months (October 2011-March 2012).

Forty baim were stocked in each of three indoor cisterns (2.33m×1.34m each) having all facilities i.e. continuous water supply through porous plastic pipes for aeration, inlet, outlet and shelter. The fish were fed with 3 different supplementary feeds as used for bagair. The highest growth (67.70±16.49g) was found in the fish fed with trash fish and the lowest growth (51.47±10.97g) was observed where Mega feed was applied for a period of 6 months (October 2011-March 2012).

Study of gonadal maturity through histological observation

Yolk vesicle (Fig. 6) and perinucleolar stage oocytes were observed in the ovaries of mohashol in the month of October 2011 and March 2012 respectively. Perinucleolar stage oocyte was observed in the month of October 2011 in the ovary of bagair. Chromatin nucleolar, perinucleolar and yolk granule stage oocytes were found in the month of October 2011 and June 2012 respectively in the ovaries of baim.

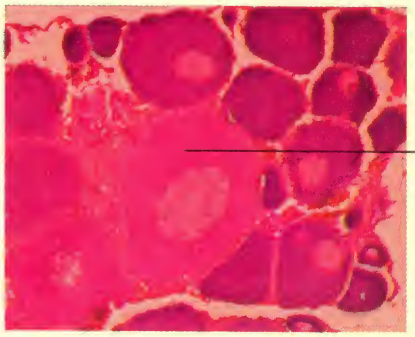

Fig. 6 Yolk vesicle stage oocyte (UVO) in the ovary of mohashol in the month of October 2011

Induced breeding

Induced breeding trial on baim *(Mastacembelus armatus)* was conducted using carp pituitary gland (PG) extract. Mature males were comparatively large in size, dark in colour and milt was available following stripping while mature females were comparatively small in size, light in colour having soft and swollen abdomen. PG dose of 40mg/kg body weight precipitated ovulation and successful stripping of ovulated eggs was possible (Fig. 7). Males were treated with PG extracts at the rate of 10mg/kg body weight once at the time of 2nd injection of the female. The first dose (30%) and the second dose (70%) were administered 6 hrs apart. The fertilized eggs were found to be adhesive, sticky, demersal and brownish in colour and 1.8±0.07mm in diameter. Embryonic development of baim proceeded up to gastrula stage (Fig. 8).

A number of induced breeding trial on mohashol *(Tor tor)* was also conducted but ovulation of eggs could not be achieved (Fig. 9).

Fig. 7 Collection of ovulated eggs through stripping of baim

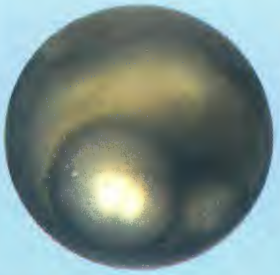

Fig. 8 Gastrula stage of embryonic development of baim

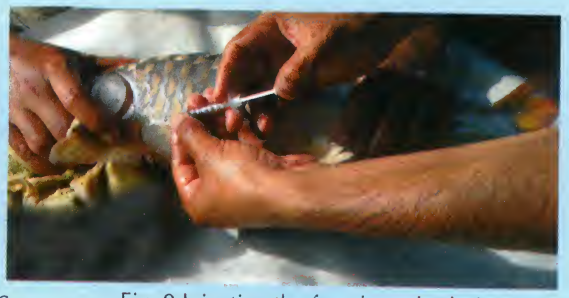

Fig. 9 Injecting the female mohashol

Achievements at a glance

Indian major carps	1. Growth performance of catla, rohu and mrigal of the Halda is the best.
	2. Riverine populations contained better genetic quality than hatchery populations.
	3. Higher genetically variable rohu individuals were observed in the Padma and the Halda populations compared to the Jamuna population.
	4. Cryopreservation protocols of IMCs (rohu and mrigal) spermatozoa were developed.
	5. Fry were produced using cryopreserved sperm through developed protocol.
	6. F_1 generation of rohu and mrigal were produced successfully through selective breeding.
Threatened fishes	1. Collection and domestication of threatened species (mohashol, bagair and baim) have been possible.
	2. Breeding seasons of mohashol and baim have been identified.
	3. Successful ovulation and fertilization were achieved and embryonic development proceeded up to gastrula stage in case of baim.
	4. Breeding trial with mohashol ended without mentionable success.

Lessons learned

- Seeds having appropriate genetic quality show significantly better growth
- Cryopreservation of IMCs spermatozoa possible
- Domestication of threatened species (mohashol, bagair and baim) possible
- Breeding protocol development of threatened species seemed possible
- Time is a factor for achieving useful breeding protocol of concerned threatened fishes

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